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Photodynamic Therapy of Facial Squamous Cell Carcinoma in Cats Using a New Photosensitizer

Michael L. Magne, DVM, MS,[2]* Carlos O. Rodriguez, DVM,[4] Susan A. Autry, BSD,[3] Benjamin F. Edwards, PhD,[1] Alain P. Theon, DVM, PhD,[2] and Bruce R. Madewell, DVM, MS[2]

[1]Center for Companion Animal Health, University of California
[2]Department of Veterinary Surgical and Radiological Sciences, University of California
[3]Department of Neurological Surgery, University of California
[4]Veterinary Medical Teaching Hospital, University of California, Davis 95616

Background and Objective: **Photodynamic therapy has been shown to be an effective treatment modality for surface-oriented neoplasms of the skin, respiratory, gastrointestinal, and urogenital systems. The purpose of this study was to assess the safety and efficacy of photodynamic therapy using a new photosensitizer in the treatment of squamous cell carcinomas of the feline facial skin.**
Study Design/Materials and Methods: **Cats with naturally occurring squamous cell carcinomas of the facial skin were entered into the study. Tumors were staged using a modification of the World Health Organization (WHO) system for classification of feline tumors of epidermal origin. Photodynamic therapy was delivered to the tumors using an argon-pumped dye laser 24 hours after the administration of the photosensitizer pyropheophorbide-alpha-hexyl-ether (HPPH-23). Following treatment, tumors were evaluated for complete response rates and local control durations.**
Results: **Fifteen tumors were staged T1a (< 1.5 cm diameter, noninvasive), 18 T1b (< 1.5 cm, invasive), and 28 T2B (> 1.5 cm, invasive). Complete response rates as well as local control durations were significantly ($P < 0.05$) related to stage. Complete response was achieved in 100% of T1a tumors, 56% of T1b tumors, and 18% of T2b tumors. One-year local control rates were 100% for T1a tumors and 53% for T1b tumors; overall 1-year local control rate for all treated tumors was 62%. Clinical, hematological, and biochemical evidence of toxicity was not seen in any cat following drug administration. However, morbidity was observed following treatment of large, invasive tumors of the nasal plane.**
Conclusion: **Photodynamic therapy with the photosensitizer HPPH-23 was safe and effective in treating early stage squamous cell carcinomas of the feline nasal plane and facial skin. How-**

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*Correspondence to: Dr. Michael L. Magne, Department of Veterinary Surgical and Radiological Sciences, University of California, Davis, CA 95616.

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Key words: feline; squamous cell carcinoma; pyropheophorbide; photodynamic therapy; photosensitizers

INTRODUCTION

The most widely used photosensitizing agents in preclinical studies have been porphyrins, chlorins, and phthalocyanines. Porfimer sodium (Photofrin, Quadra Logic Technologies), a porphyrin compound, is currently the only commercially licensed photosensitzer available, and there are numerous reports of photofin-based photodynamic therapy (PDT) in human patients with neoplasms of the skin, gastrointestinal, respiratory, and urogenital systems [1–3]. PDT using porfimer sodium also has been applied to various neoplasms in dogs and cats [4]. Limitations of porfimer sodium as a tumor therapeutic agent include limited tissue penetration due to its short (630 nm) activation wavelength and prolonged cutaneous photosensitization of patients to sunlight. There is considerable interest in developing photosensitizers that overcome these limitations. Phthalocyanine compounds are activated at a longer wavelength (675 nm), and preliminary favorable results following treatment of feline squamous cell carcinoma have been reported with chloraluminum-sulfonated phthalocyanine-based PDT [5].

The purpose of this study was to investigate the efficacy and toxicity of a new chlorophyll A derivative photosensitizer, pyropheophorbide-alpha-hexyl-ether (HPPH-23) for photodynamic therapy of cats with naturally occurring squamous cell carcinomas of the facial skin. HPPH-23 has an activation wavelength of 665 nm and, based on murine studies, is very rapidly cleared from normal skin, thus potentially sparing normal tissues from photosensitization effects. Other possible advantages of HPPH-23 as a photosensitizer include its pure, clearly defined chemical structure and a higher extinction coefficient at its absorption maximum compared to porfimer sodium [6].

MATERIALS AND METHODS

Tumors

Naturally occurring squamous cell carcinomas of the nasal plane and/or facial skin of client-owned cats were entered in the study. For entry into study, cats were determined to be free of other major systemic diseases based on physical examination, hematology, serum biochemistry, and serology for retroviruses. Neoplasms were histologically confirmed and clinically staged using a modification of the WHO TNM system for feline tumors of epidermal origin [7]. For purposes of this study, tumors were categorized into four groups. The first group (T1a) was comprised of noninvasive lesions measuring < 1.5 cm in diameter. The second group (T1b) contained invasive neoplasms < 1.5 cm in diameter. The third group (T2a) consisted of noninvasive neoplasms > 1.5 cm in diameter, and the fourth group (T2b) was represented by invasive neoplasms measuring > 1.5 cm in diameter. Invasiveness was determined by histopathology and visual assessment. All cats were free of regional and thoracic metastases on the basis of palpation or thoracic radiographs. Sixty-one tumors on 51 cats were treated. There were 15 tumors staged T1a, 18 T1b, and 28 T2b. Forty-three tumors were on the nasal plane (12 T1a, 16 T1b, 15 T2b), and 18 involved facial skin, usually eyelids and ear pinnae (3 T1a, 2 T1b, 13 T2b).

Study Design

The first treatment group consisted of tumors of all stages (T1a, T1b, T2b) that were treated with a single PDT to evaluate efficacy. Treatment group 2 consisted of T1b and T2b lesions that received two or three PDT treatments; the intent of the group 2 study was to evaluate cumulative toxicity and to determine if efficacy could be improved with multiple treatments. There was no strict a priori schedule established for second and third PDT treatments; rather, patients were selected for treatment when there was visible evidence of tumor persistence/recurrence. Second and third treatments were administered following the same protocol as outlined below.

Photosensitizer

HPPH-23, as previously described [7], was kindly provided by Dr. Thomas J. Dougherty (Roswell Park Cancer Institute, Buffalo, NY) in an aqueous solution (0.1% Tween, 2% ethanol, 5% dextrose in water, pH = 7.5) and was stored in a

lightproof container at 4°C until use. HPPH-23 is a pheophorbide photosensitizer with an extinction coefficient of 50,000 $M^{-1}cm^{-1}$ at its major visible absorption peak of 665 nm. For the purposes of this study, an investigational new animal drug (INAD) permit was obtained from the Food and Drug Administration. Prior to use in client-owned animals, acute toxicity, pharmacokinetic, and tissue distribution studies for HPPH-23 were performed in 22 clinically healthy adult cats (data not included). No evidence of clinical, hematologic, or biochemical toxicity was observed in cats given intravenous doses at 0.3, 0.6, 0.9, and 1.2 mg/kg, and these data suggested that HPPH-23 at a dosage of 0.3 mg/kg was safe for administration to healthy adult cats.

Light Delivery System

An argon pumped dye laser (Spectra Physics 2040 and 275B, Mountain View, CA), tuned to emit light at 665 nm wavelength, was used. A quartz fiber fitted with a microlens (Laser Therapeutics, Buellton, CA) was used to ensure uniform light delivery. Laser output was monitored before and after each treatment with a power meter (Photodyne, 66XLA, Newbury Park, CA).

Treatment Protocol

HPPH-23 was administered intravenously at a dosage of 0.3 mg/kg 24 hours prior to irradiation. Inhalation anesthesia using isofluorane was used for all treatments. Tumors were irradiated at a wavelength of 665 nm, an energy density of 100 J/cm^2, and a power density of 75 mW/cm^2. The light spot was centered at the midpoint of the tumor's largest diameter and extended 2–5 mm beyond the visible lesion. The eyes and facial skin outside the treatment field were shielded from incident laser light. Duration of therapy for all tumors was 22 minutes, 15 seconds. Treatment protocol was determined based on the normal cat toxicity study, personal communications with the photosensitizer supplier, and data from rodent studies showing an optimal therapeutic index at a dose of 0.3 mg/kg followed by photoillumination at 24 hours. Cats were discharged to owner care 24–48 hours following therapy.

For analgesia, oxymorphone was administered at a dosage of 0.1 mg/kg subcutaneously to all cats at the end of irradiation. Dexamethasone sodium phosphate, 1.0 mg/kg intravenously, was administered immediately following the conclusion of treatment and 6 hours later. Prednisolone was given orally at 1.0 mg/kg/day for 7 days in those cats with posttreatment nasal swelling, dyspnea, and anorexia.

Assessment of Response

Cats were examined 2, 4, 8, 12, 24, 36, and 52 weeks after treatment. Follow-up data for intervals > 52 weeks was collected by direct examination in some patients or by verbal telephone communication, either with the animal owner or the referring veterinarian. Mean and median follow-up intervals were 9.0 and 9.7 months, respectively.

Complete response (CR) was defined as complete regression of the lesion with re-epithelialization; partial response (PR) as 50% or more reduction in tumor diameter; < 50% reduction in tumor diameter or progressive disease was considered no response (NR). These end-points reported herein were determined at 3 months posttherapy and lasted a minimum of 4 weeks. Local control of disease was defined as absence of tumor recurrence after a complete response was achieved, and local control data were computed from the date of treatment. Likewise, cats not achieving complete response were considered treatment failures on day 1.

Local or systemic treatment side effects were determined by physical examinations and client verbal reports. Additional hematologic, serum biochemical, radiographic, or ultrasonographic evaluations were made if deemed clinically necessary. Postmortem necropsy examinations were done on cats that died or were euthanatized during the study period.

Statisical Analysis

Initial response (CR) rates were analyzed using X^2 tests. Local control rates were estimated by the product-limit method [9]. Differences in local control durations were tested for statistical differences using the Breslow [10] and log-rank test statistics [11].

RESULTS

Overall, treatment resulted in CR in 49% (30/61), PR in 12% (7/61), and NR in 39% (24/61) of the feline facial squamous cell carcinoma.

CR was achieved in 100% (15/15) of the T1a tumors following a single therapy session. CR was achieved in 56% (10/18) of the T1b tumors following a single treatment session. Of the T1b tumors, 11% (2/18) were considered PR following a single therapy, and 33% (6/18) were classified as NR. CR

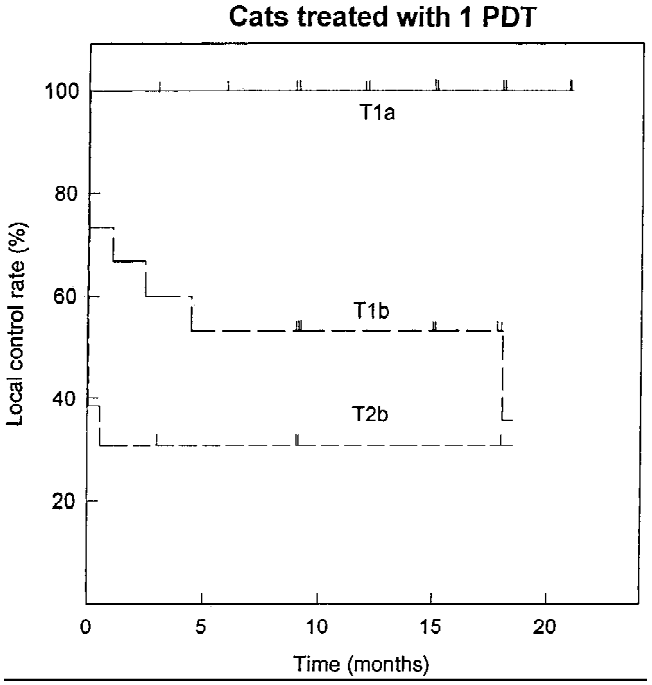

Fig. 1. Kaplan-Meier curve comparing disease progression-free intervals for T1a, T1b, and T2b tumors receiving a single PDT treatment.

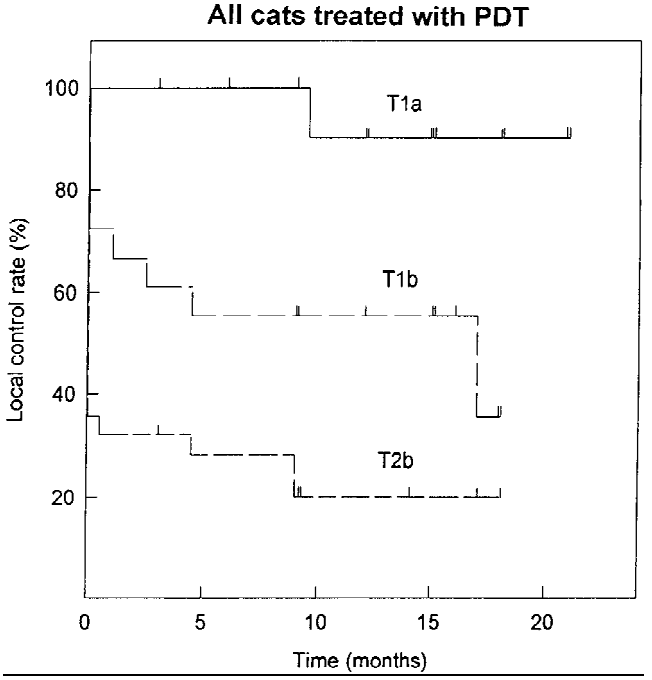

Fig. 2. Kaplan-Meier curve comparing disease progression-free intervals for all treated tumors.

was achieved in 18% (5/28) and PR in 18% (5/28) of the T2b tumors following treatment, whereas 64% (18/28) of the T2b tumors were considered nonresponders. Of the T2b nonresponders, 50% (9/18) were classified PR at 4-week evaluations, but had progressive disease and were deemed treatment failures at either the 8- or 12-week evaluations. Initial CR rates were significantly associated with tumor stage (Chi square = 29.1; $P < 0.0001$).

Local control duration was also significantly affected by tumor clinical stage, both in tumors receiving a single PDT therapy (log-rank $P = 0.0005$; Breslow $P = 0.0022$) as well as tumors receiving multiple (2–3) treatments (log-rank $P < 0.0001$; Breslow $P < 0.0001$) (Figs. 1, 2). For tumors treated with a single PDT session, the overall 1-year local control rate (± S.E.) was 61.7 (± 7)%. By stage, 1-year local control rate was 100% for T1a tumors and 53.3 (± 13)% for T1b tumors. A 1-year local control rate could not be computed for T2b tumors undergoing a single treatment as only 1 tumor remained CR at 1 year.

For tumors receiving from two or three PDT treatments, the overall 1-year local control rate was 47.6 (± 6)%. By stage, 1-year local control rates were 90 (± 9)% for T1a tumors, 55.6 (± 12)% for T1b tumors, and 20 (± 7)% for T2b tumors. No significant ($P > 0.05$) difference was observed in local control rates for tumors receiving 1 vs. 2 or 3 treatments.

Acute skin reactions were observed in all cats and included erythema and edema beginning during or immediately after the treatment session. Edema often extended beyond the treatment field and in cats with large nasal plane lesions, often resulted in stertor and dyspnea. These acute tissue reactions subsided in all cats within 3–5 days of therapy. Anorexia was also observed in cats following treatment of large nasal plane lesions and typically resolved within 7 days. Post-treatment nasal obstruction and dyspnea were reported in 15 cats, all within the T1b and T2b tumor groups. Anorexia ensued in 26 cats, affecting cats with comparable frequency in the T1a (6 cats, 50%), T1b (9 cats, 56%), and T2b (11 cats, 73%) tumor groups. Anorexia and dyspnea were reported in only one cat with a nonnasal plane (periocular), T2b lesion.

Tumor necrosis and formation of a scab at the treatment site were observed within 2 weeks following treatment, and tissue slough occurred between 2 and 8 weeks. Secondary bacterial infection of the treatment site occurred in 50% of

the tumors during this 2–8-week period, but this complication was easily controlled with antibiotics. Although owners were instructed to shelter cats from sunlight during the immediate post-treatment period, mild cutaneous photosensitization resulted in two cats following sunlight exposure within 2 weeks of treatment. This reaction was characterized by erythema, pruritus, and slight hair loss involving the head and dorsal midline. The only appreciable chronic toxicity noted was alopecia within the treatment field in all treated cats.

Cardiovascular complications were observed in five cats immediately following HPPH-23 based PDT of nasal plane lesions, all within the T2b tumor group and following a single treatment. This was characterized by the peracute development of severe dyspnea and pulmonary edema in five cats and pleural effusions in three cats. Heart murmurs had been detected in two of these cats before treatment; however, they were considered free of clinically relevant cardiac disease based on thoracic radiography, cardiac ultrasonography, and electrocardiography. Treatment for congestive heart failure was used in all five cats; clinical recoveries were complete in four cats, whereas one cat required long-term management for heart failure. Occurrence of these acute, systemic toxicities was unrelated to single versus multiple treatment sessions, i.e., there was no apparent cumulative toxicity.

Eight cats died or were euthanatized during the period of study. Two cats were euthanatized due to treatment failure and tumor progression. Two cats were euthanatized due to progressive deterioration in general health; postmortem and histopathologic examinations in these two cats were consistent with systemic Aspergillosis. Four cats died or were euthanatized because of unrelated illnesses.

DISCUSSION

Our results indicated that HPPH-23 based PDT is an effective therapy with minimal toxicity for cats with early stage (small, noninvasive) squamous cell carcinomas of the nasal plane and facial skin. Although there are limited data on the results of therapy for cats with these neoplasms, our results compare favorably to those reported in the veterinary literature using surgery, radiation therapy, or PDT with other photosensitizers [12–19]. One advantage of PDT over conventional radiation therapy is the ability to eradicate certain lesions with a single treatment, thereby reducing overall treatment duration as well as number of anesthetic episodes required for patient management (Figs. 3–5). Additionally, PDT is less cosmetically deforming than surgical excision. Complications following PDT for cats with small lesions are minimal, and these reactions can be easily managed symptomatically. Large, invasive tumors, however, are difficult to control using any treatment method. In the present study, the complete response and local control rates for such tumors, as well as the acute side effects, were clinically unacceptable.

HPPH-23 appears to be a safe compound for parenteral administration in cats, and no adverse systemic effects of the drug were observed in 22 clinically healthy cats (data not shown) or in 51 tumor-bearing cats following its intravenous administration. However, HPPH-23 appears to be a potent photosensitizer in cats, based on the degree of tissue inflammation and edema that follows HPPH-23-based PDT. These acute reactions were more profound than those previously observed following phthalocyanine-based PDT [5] or those described in cats following porfimer sodium-based PDT [16]. The cardiovascular decompensation observed was limited to cats with large and invasive (T2b) lesions of the nasal plane. It is conceivable that this complication is a sequela to the intensity of the inflammatory response following HPPH-23 based PDT. The PDT-induced inflammatory response is attributed, at least in part, to the release of potent vasoactive mediators such as prostaglandins, leukotrienes, thromboxane, histamine, and cytokines such as interleukins, interferons, and tumor necrosis factor [20,21]. If sufficiently increased in the systemic circulation following PDT, these vasoactive compounds may exert a deleterious influence on cardiovascular homeostasis. A lethal cardiovascular collapse phenomenon also has been reported following porfimer sodium-based PDT in mice with large

Fig. 3. Photograph demonstrating a large, T2b squamous cell carcinoma involving the right medial canthus and inferior palpebra prior to therapy.

Fig. 4. The same neoplasm depicted in Figure 3, 4 weeks following photodynamic therapy.

Fig. 5. The same neoplasm depicted in Figures 3 and 4, 8 weeks following photodynamic therapy. Note the alopecia within the treatment field.

Figs. 3–5

experimentally induced tumors [22]. Hematologic and histopathologic abnormalities in affected mice represented a form of traumatic shock, and the cardiovascular complications could be inhibited by administration of cyclo-oxygenase inhibitors (indomethacin, aspirin), anticoagulants (warfarin), or antihistamines. Similar cardiovascular complications have not been observed in cats with smaller nasal plane lesions following HPPH-23-based PDT.

Local edema was observed in all cats following treatment, and in many cats, edema extended distant from the treatment field. Edema following PDT also has been attributed to the release of vasoactive compounds. Anorexia and dyspnea were also common sequelae to treatment of cats with nasal plane lesions, and the severity of these complications paralleled the size of the lesion. Cats were routinely administered corticosteroids following PDT in an attempt to reduce local tissue reactions and maintain appetite. The administration of corticosteroids following irradiation also has been shown to enhance the antitumor effects of PDT [23]. Secondary bacterial infection was a common complication in cats following PDT, although it was easily controlled with empirical antibiotic therapy. The tissue necrosis observed following HPPH-23 based PDT occurred uniformly within treatment fields, i.e., there did not appear to be substantial "sparing" of adjacent normal tissues.

The causes for the development of aspergillosis in two cats remain unclear. It is our assumption that photodynamic therapy in conjunction with administration of corticosteroids compromised immunologic integrity [24], thereby allowing establishment of an opportunistic infection. Contamination of HPPH-23 vials as a source of *Aspergillus spp.* was excluded by negative fungus cultures.

The morbidity associated with HPPH-23-based PDT in cats with large, invasive, nasal plane lesions, as reported here, was unacceptable. Possible means to prevent these complications include more critical pretreatment cardiovascular evaluation, limiting treatments to smaller tumor volumes, perhaps expedited by partial resection of the neoplasm before PDT, and a more thorough understanding of the inflammatory mediators evoked by PDT, thus allowing more specific pharmacologic interventions.

Squamous cell carcinoma of the facial skin of cats serves as a useful model for development of PDT technology. This study confirms that PDT is very effective for superficial lesions. Multiple (up to 3) treatments may not improve efficacy. However, multiple therapies were not associated with increased toxicity, thus dose fractionation might be used to decrease acute side effects. Optimization of PDT for squamous cell carcinomas of the feline nasal plane and facial skin may thus serve as a prelude to more widespread application of PDT to tumors at other anatomic sites or of other histologic types.

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